

## DATA EVALUATION RECORD

1. Chemical: PP321 = *Lambda cyhalothrin*
2. Test Material:  $^{14}\text{C}$ -Cyclopropane-labeled PP321  
92.8% ai
3. Study Type: *Daphnia magna* Life Cycle (21-Day Renewal)  
Chronic Toxicity Test

Test Species: *Daphnia magna*

4. Study ID: Hamer, M.J.; Farrelly, E.; Hill, I.R. (1985)  
PP321: 21-Day *Daphnia magna* Life Cycle Study.  
Submitted by ICI Americas, Inc.; prepared by  
ICI, Plant Protection Division, Jealotts Hill  
Research Station, Bracknell, Berkshire; EPA  
Accession No. 073989.

5. Reviewed By: Candy Brassard  
Environmental Protection  
Specialist  
EEB/HED

Signature: *Candy Brassard*

Date: *9/29/87*

6. Approved By: Douglas J. Urban  
Head, Section III  
EEB/HED

Signature: *Douglas J. Urban*

Date: *10/3/87*

7. Conclusion:

*upgraded to Supplemental*

This study is classified as ~~invalid~~. Many discrepancies were outlined in section 14 of this review. The major concerns are as follows:

- The study author reported selecting the healthiest female daphnids on day 6 of the study, and reducing the number of daphnids from 50 to 30 in each test chamber.
- The study design only included two test chambers per treatment level, instead of seven test chambers (with one daphnid each) and three test chambers (with five daphnids each). These discrepancies cause concern for the scientific soundness and therefore the study is classified as "Invalid."

8. Recommendations:

EEB recommends that the study be conducted again, preferably under flow-through conditions and following the Standard Evaluation Procedure (SEP), *Daphnia magna* Life Cycle (21-day Renewal) Chronic Toxicity Test, M. Rexrode and T. Armitage (July 1986) EPA 540/9-86-141.



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9. Background Information:

This study was submitted to support registration of a new synthetic pyrethroid, Karate or PP321.

10. Discussion of Individual Test: N/A

11. Materials and Methods:

a. Test Animals - Daphnia magna < 24 hours old were taken from cultures maintained at Jealotts Hill. The test organisms were cultured in dechlorinated mains water and maintained on a diet of yeast and Chlorella vulgaris at  $20 \pm 1$  °C on a 16-hour day.

b. Test System - Test solutions were replaced every 12 hours. The study author found that the flow-through method had failed to maintain a satisfactory series of concentrations of test material for the duration of the study.

Duplicate test vessels consisted of 3 L glass beakers containing 2 L of test solution. The vessels were maintained at  $20 \pm 1$  °C in a recirculating water bath. Light intensity was 800 lux with a 16 hr light/8 hr dark photoperiod.

c. Study Design - (Excerpted from submission)

"50 Daphnia (less than 24 hours old) were introduced to each test chamber at the start of each test. On day 6, the number in each chamber was reduced to 30 by selecting the healthiest females and discarding the remainder. Adult Daphnia were transferred to fresh test solutions in a clean beaker every 12 hours using a glass pipette. After transfer of the adults, any young Daphnia were removed by sieving the test solution through a fine nylon mesh and transferring them to the fresh solution. Daphnia were fed twice daily throughout the study on a Chlorella vulgaris/yeast diet, each feed consisting of 4 ml of a Chlorella suspension ( $10^8$  cells ml<sup>-1</sup>) plus 4 ml of an active dried yeast suspension (2 mg ml<sup>-1</sup>). The food was added to each new test solution after its preparation. Test chambers were gently aerated to aid suspension of the food.

"Assessments were made throughout the study of Daphnia survival, growth and reproduction. Survival of the adult Daphnia was recorded each time they were transferred to fresh solutions. Lengths (top of head to base of spine) of adult Daphnia were measured, using a graticule lens in a stereomicroscope, on day 9; by randomly selecting 10 individuals for observation, and then carefully returning them to the test chambers. On day 21 all remaining females were measured.

"Three times weekly (Monday, Wednesday and Friday mornings), the young Daphnia were removed from the test chambers by sieving, resuspended in dechlorinated water, counted for live and dead young and then discarded.

"On day 13 of the test twenty young (first instar) Daphnia, were removed from each test chamber and maintained in dechlorinated water. They were fed algae and yeast daily. Survival of these Daphnia initially added and their young was determined after 7, 10 and 13 days, to look for any effects."

The DO and pH were measured in all test chambers three times weekly. On days 0, 7, 14, and 21, the DO and pH were measured in the 12-hour test solutions just prior to being replaced. The temperature of the water bath was recorded every 12 hours.

(Excerpted from submission)

"During this study representative test solutions were sampled for quantification of total radioactivity and also for characterization of the radioactivity; to determine whether hydrolysis or isomerisation had occurred. In addition, representative acetone stock solutions (used to prepare the test concentrations) were analysed by TLC and by HPLC."

#### Measurement of Concentrations

- Total Radioactivity - Water samples were taken for LSC at the end of the 12-hour period on days 0, 7, 14, and 21 of the study in order to measure the  $^{14}\text{C}$ -residues.
- TLC was used to resolve PP321 and its hydrolysis product. On days 0, 7, 14, and 21, samples of 40 and 20 ng/L were measured at the beginning and the end of the 12-hour period.

In addition, acetone stock solutions were analyzed by TLC on days 0, 15, and 21.

High-performance liquid chromatography (HPLC) was used to resolve PP321 from the other isomers of PP564 and to quantify the extent of isomerisation. Water samples from 40 and 20 ng/L were analyzed on days 0, 7, and 14. On day 21, only the 20 ng/L sample was analyzed (the 40 ng/L rate was not prepared due to 100% mortality of the Daphnia). Each solution was analyzed immediately after preparation and at the end of the 12-hour period.

On days 3 and 7 of the study, a 5 ng/l sample was extracted (five samples total) at 0 and 12 hours. On days 16 to 21, the 2.5 ng/l sample was extracted at 0, 9, and 12 hours. For the TLC and HPLC, 1 L aliquots of each solution were taken and extracted with dichloromethane and hexane, respectively.

d. Statistics - The following parameters were subjected to ANOVA with paired test chambers as replicates:

- Total number of young produced;
- Length of adults on days 9 and 21; and
- Number of young per female.

The number of young/female was calculated by dividing the total young produced by the number of "female reproductive days" for each test chamber.

The number of female reproductive days was calculated from a summation of the number of surviving females each day, from day 6 when reproduction started to the end of the study (day 21). The maximum number of female reproductive days in a test chamber was 450 (30 surviving females each day for 15 days).

## 12. Reported Results:

The DO levels were > 7.9 mg/L (86% saturation) at all measurement times. The pH ranged from 7.7 to 8.4. The temperature of the water bath remained at  $20 \pm 1$  °C throughout the test.

Results of Analysis of Concentrations - See Table 9 for summary of  $^{14}\text{C}$  residue analysis.

Table 8 summarizes the measured concentrations at the beginning of the test for all treatment levels. Table 9 for radiochemical concentrations at 0 and 12 hours on days 0, 7, 14, and 20.

TLC and HPLC - Tables 10 to 13 include the results of the TLC and HPLC.

Biological Results - See Table 5 for statistical analyses. Tables 14 to 17 list the full results.

(Excerpted from submission)

"There were significantly fewer female reproductive days in the 40 and 20 ng  $\text{l}^{-1}$  concentration (see Tables 5 and 14). At 10 ng  $\text{l}^{-1}$  and below there was no significant effect on the survival of the adult Daphnia. By the end of the study, 98% of the Daphnia surviving at day 6 (after

sorting) had died in the two 40 ng l<sup>-1</sup> chambers. The respective figure in the 20 ng l<sup>-1</sup> chambers was 62%. In other test concentrations, deaths were in the range 8-22% (10% in the controls).

"Analysis of the Daphnia lengths on day 9 show that only at 40 ng l<sup>-1</sup> were the Daphnia significantly smaller than the controls (see Table 16). At day 21 (see Tables 5 and 17), when the 40 ng l<sup>-1</sup> concentration was omitted from the analysis (only one Daphnia remaining), there was no significant difference in length between the other concentrations and the control.

"The number of young produced per female per reproductive day (see Tables 5, 14, and 15) showed all the test concentrations, except the 2.5 ng l<sup>-1</sup> to be significantly different to the control. The other chambers, although significantly different to the control, showed no significant differences between them. They all produced between 5.00 and 5.35 young per female per day, approximately 70% of that in the control.

"The numbers of young produced from the Daphnia transferred to and cultured in dechlorinated water for 13 days showed no significant differences in the total numbers of young produced or the number of young produced per female per day (see Appendix VI, Tables 18 and 19). In one replicate of the Daphnia from the 40 ng l<sup>-1</sup> concentration, only 6 of the original 20 Daphnia survived the 13 days."

13. Study Author's Conclusion/OA Measures:

(Excerpted from submission)

"The study demonstrates that if Daphnia are kept in maintained concentrations of PP321 for 21 days, the number of young produced per female is reduced at concentrations of approximately 5 ng l<sup>-1</sup> and above. However, the data also show that at concentrations of 5-40 ng l<sup>-1</sup>, if the Daphnia survive they reproduce at a rate only slightly below (approximately 70%) that of the control. Acute effects were only apparent in the 20 and 40 ng l<sup>-1</sup> concentrations, after 13 and 11 days exposure, respectively.

"During the conduct of this study, the Quality Assurance Unit carried out the following audits in accordance with ICI Policy of Good Laboratory Practice (Study No. PP321/CN/02)."

14. Reviewer's Discussion and Interpretation of the Results:

There were major discrepancies that detracted from the study. They are as follows:

a. Test Procedures

- The raw data were not submitted.
- The study did not indicate if the daphnids were obtained from a stock (at least 10-12 days old) that was a separate culture that was maintained for 21 days prior to test initiation.
- The study author reported that dechlorinated mains water was used for the dilution water. Dechlorinated water should not be used because removal of chlorine is rarely complete and residual chlorine can be quite toxic to aquatic organisms.
- Test tanks were aerated. The SEP guideline clearly states that the test chambers should not be aerated.
- Study design only included two test vessels per treatment level. The study should have used (a) seven beakers at each concentration with one daphnid per beaker for collection of data on survival, growth, and reproduction; and (b) three beakers at each concentration with five daphnids in each for collection of data on survival only. Assignments of daphnids should be randomized. The test begins when test organisms are first placed in the test solution.
- In addition, both the solvent control and the control should be replicated the same as the treatment levels. This study did not report if replicates were used for the controls.
- The study author reported that on day 6, the number in each chamber was reduced to 30 (from 50) by selecting the healthiest females and discarding the remainder. Selecting the healthiest test organisms makes the study results biased; therefore, the practice is considered to be scientifically unsound.
- It appears that the data for the number of young produced, female reproductive days, and daphnia lengths for both the solvent control and the control were pooled. This is unacceptable. (See Table 5.)
- The water hardness was reported to have been as high as 275 mg/L. It should have only been 160 to 180 mg/L.

- It appears from Table 9 that the measured concentrations were as low as 8% of the nominal. And, in some instances, the measured concentrations were considerably higher than the nominal (4.5 times). The study author should be aware that the measured concentrations should be  $\pm 30\%$  of the nominal concentration.
  - The concentrations should be measured in each test vessel. The study reported only measuring four of the five treatment levels (using LSC) at 0-hour, not both 0 and 12 hours. All treatment levels should be measured.
- b. Statistical Analysis - Since the study design did not include enough replicates, an ANOVA was not conducted. In addition, the discrepancies found in the study caused concern for the statistical validity of the study.
- c. Discussion of Results - To select for the healthiest test organisms is scientifically unsound. This study reported that the healthiest females were chosen on day 6, and the rest were discarded. This makes the study results biased.

In addition, the study design only used duplicates for the treatment levels. We recommend at least 7 test chambers with one daphnid in each and three test chambers with five daphnids in each. In addition, the study design did not indicate if replicates were used for both the control or solvent control.

The renewal method is an acceptable practice for assessing the toxicity of a chemical to daphnids. However, since the chemical being tested is a synthetic pyrethroid, we recommend flow-through conditions for future testing.

d. Adequacy of the Study

- 1) Classification - Invalid *upgraded to Supplemental*
- 2) Rationale - Due to the discrepancies outlined in section 14.
- 3) Repairability - The study cannot be repaired.

TABLE 4 : Mean Values for  $^{14}\text{C}$ -Residue Analysis from Days 0, 7, 14 and 20/21

Nominal concentration ( $\text{ng l}^{-1}$ )	Sample time	Mean concentration ( $\text{ng PP21 equivalents l}^{-1}$ )	TLC Analysis		HPLC Analysis			
			% Radioactivity in water dichloromethane extract after rotary evaporation	% Radioactivity applied to TLC plates co-chromatographing with PP21	% Radioactivity in water hexane extract after rotary evaporation	% Radioactivity injected, eluted off the column	% Radioactivity eluted from the column in fractions	
							A + A'	B' B total remainder
40	0 hrs	40.1 <sup>a</sup>	92 <sup>c</sup>	94	97 <sup>d</sup>	86	5.9	3.8 87.6 1.6 1.0
	12 hrs	25.1 <sup>a</sup>	60 <sup>c</sup>	74	54 <sup>d</sup>	86	28.8	2.4 63.0 1.4 4.1
20	0 hrs	19.0 <sup>b</sup>	107 <sup>c</sup>	88	92 <sup>e</sup>	87	5.7	3.0 86.5 1.5 4.0
	12 hrs	13.4 <sup>b</sup>	63 <sup>c</sup>	76	49 <sup>e</sup>	99	28.7	2.1 58.5 3.0 7.6

a Mean of 7 values (2 replicates on each of days 0, 7, 14 and one sample on day 21)

b Mean of 8 values (2 replicates on each of days 0, 7, 14 and 21)

c Mean of 4 values (1 replicate on each of days 0, 7, 14 and 21)

d Mean of 3 values (1 replicate on each of days 0, 7, 14 and 21)

e Mean of 4 values (1 replicate on each of days 0, 7, 14 and 21)



**TABLE 5 : Biological Assessments : Statistical Analyses**

Nominal Concentration (ng l <sup>-1</sup> )	Total Young Produced	Female Reproductive Days	Young Per Female Reproductive Day	Daphnia Lengths Day 9 (mm)	Daphnia Lengths Day 21 (mm)
Control	3104 A	426 A	7.28 A	4.75 A	4.87 A
2.5	2712 AB	437 A	6.20 AB	4.49 AB	4.87 A
5	2288 AC	427 A	5.35 B	4.63 A	4.92 A
10	2103 BC	417 A	5.04 B	4.54 A	4.89 A
20	1757 CD	349 B	5.00 B	4.35 AB	4.74 A
40	1041 D	200 C	5.20 B	4.09 B	
Standard Error (Single Plot)	347	13	0.72	0.18	0.09
Coefficient of Variation	16.0%	3.6%	12.7%	3.9%	1.9%
LSD P = 5%	848	33	1.77	0.43	0.24

Treatment means with no letter in common are significantly different at the 5% probability level

## APPENDIX I : Dissolved Oxygen and pH Measurements

TABLE 6 : Dissolved Oxygen Measurements ( $\text{mg l}^{-1}$ )

Nominal Concentration ( $\text{ng l}^{-1}$ )	Day	0		2	5	7		9	12	14		16	18	21	
		0hr	12 hr			0hr	12 hr			0hr	12hr			0hr	12hr
Control	I	9.1	8.6	9.1	8.8	9.1	9.0	9.1	8.6	8.6	8.9	9.0	8.9	8.7	8.9
	II	9.2	8.8	9.0	9.0	9.0	8.9	8.9	8.0	8.2	9.0	8.8	8.4	8.9	8.7
2.5	I	9.2	8.6	8.9	8.9	9.0	9.0	8.7	8.2	8.0	8.8	8.7	8.0	8.7	8.8
	II	9.2	8.6	9.0	8.9	9.0	9.1	8.8	8.0	8.0	9.1	8.7	8.1	8.4	8.7
5.0	I	9.1	8.5	9.1	9.0	9.1	8.9	8.9	8.0	8.0	8.9	8.9	8.3	8.3	8.7
	II	9.2	8.6	9.1	8.9	9.1	8.6	8.9	8.1	8.1	8.8	9.0	8.4	8.3	8.5
10.0	I	9.2	8.5	8.8	9.0	9.1	8.7	9.1	8.2	8.1	8.8	9.0	8.3	8.2	8.5
	II	9.2	8.5	9.0	9.0	9.1	8.7	9.1	8.2	8.1	8.8	9.0	8.4	8.5	8.6
20.0	I	9.2	8.4	8.9	9.0	9.2	8.9	9.0	8.1	8.2	9.0	9.1	8.3	7.9	8.7
	II	9.2	8.5	9.0	9.1	9.2	9.0	9.1	8.3	8.2	8.8	9.1	8.8	8.0	8.8
40.0	I	9.2	8.6	9.0	9.1	9.2	8.8	9.2	8.1	8.4	8.9	9.2	8.7	8.1	8.8
	II	9.2	8.6	9.1	9.0	9.2	8.6	9.2	8.4	8.4	8.9	9.2	-	-	-

- no measurement taken

TABLE 7 : pH Measurements

Nominal Concentration ( $\text{ng l}^{-1}$ )	Day	0		2	5	7		9	12	14		16	18	21	
		0hr	12hr			0hr	12 hr			0hr	12hr			0hr <sup>a</sup>	12hr
Control	I	8.2	8.3	8.1	8.0	8.0	8.1	8.0	8.2	8.1	7.9	7.9	8.0	-	8.2
	II	8.2	8.3	8.1	8.0	8.1	8.2	8.0	8.1	8.0	8.0	7.8	7.9	-	8.1
2.5	I	8.1	8.3	8.1	8.0	8.0	8.1	8.0	8.2	8.1	8.2	7.9	7.9	-	8.2
	II	8.2	8.3	8.2	8.0	8.0	8.1	8.0	8.2	8.0	8.2	7.9	8.0	-	8.2
5.0	I	8.1	8.4	8.1	8.0	8.0	8.1	8.0	8.3	8.0	8.2	7.9	8.0	-	8.2
	II	8.0	8.3	8.0	7.9	7.8	8.1	8.0	8.1	8.0	8.2	7.9	8.0	-	8.1
10.0	I	8.1	8.2	8.1	7.9	8.0	8.2	8.0	8.2	8.2	8.2	7.9	8.1	-	8.1
	II	8.1	8.3	8.1	7.9	8.0	8.2	8.0	8.2	7.9	8.2	7.9	8.0	-	8.2
20.0	I	8.0	8.4	8.1	7.9	8.0	8.2	7.9	8.2	8.0	8.2	7.8	7.9	-	8.1
	II	8.1	8.2	8.1	7.9	7.8	8.2	8.0	8.3	8.1	8.1	7.8	7.7	-	8.2
40.0	I	7.9	8.2	8.0	7.9	7.8	8.1	8.0	8.2	7.8	8.1	7.8	8.0	-	8.1
	II	8.0	8.2	8.1	8.0	7.7	8.0	7.9	8.2	7.9	7.9	7.8	-	-	-

<sup>a</sup> not done due to machine failure

- no measurement taken

## APPENDIX III : Characterisation of Radioactivity in Test Solutions

TABLE 11 : Determination of Isomer Ratios Throughout the Study

Sample Nominal Concentration (ng l <sup>-1</sup> ), Study Day, Age of Solution Sampled	% of Total Radioactivity in Water			% Recovery of Radioactivity off HPLC column	% of Radioactivity Eluted from HPLC Column in Fractions			
	in hexane after extraction	in hexane after rotary evaporation	remaining in water after extraction		A'+ A	B'	B	total trans remainder
40II, Day 0, 0 hrs	110	107	1	86.2	1.1	3.2	93.9	1.6
40II, Day 0, 12 hrs	46	52	30	78.7	29.5	2.7	66.6	1.0
20II, Day 0, 0 hrs	105	99	-	91.6	1.3	5.2	91.2	1.8
20II, Day 0, 12 hrs	56	41	26	110.4	26.5	2.2	65.8	2.6
40II, Day 7, 0 hrs	100	88	5	86.0	8.4	2.6	86.4	1.5
40II, Day 7, 12 hrs	54	56	28	88.8	30.9	2.7	55.3	1.0
20II, Day 7, 0 hrs <sup>a</sup>	114	26 <sup>a</sup>	2					
20II, Day 7, 12 hrs	53	53	37	113.2	29.3	2.6	62.1	5.2
40II, Day 14, 0 hrs	103	95	-	85.3	8.2	5.6	82.4	1.8
40II, Day 14, 12 hrs	43	53	31	91.9	26.0	1.9	67.0	2.2
20II, Day 14, 0 hrs	90	85	4	73.2	7.4	1.6	79.0	0.9
20II, Day 14, 12 hrs	50	49	37	68.3	24.0	1.7	48.4	2.2
20II, Day 20, 0 hrs	82	92	3	96.8	8.4	2.2	86.2	1.8
20II, Day 21, 12 hrs	56	52	23	102.9	35.1	2.0	57.6	1.6
5I, Days 3-7, 0 hrs				83.7	5.1	2.5	82.4	1.4
5I, Days 3-7, 12 hrs				86.3	32.2	2.2	63.4	0.4
2.5I, Days 16-21, 0 hrs				90.9	12.5	4.3	79.0	2.0
2.5I, Days 16-21, 12 hrs				89.1	30.4	1.2	64.9	1.5

<sup>a</sup> sample split during rotary evaporation

## APPENDIX V : Biological Results

TABLE 14 : Numbers of Surviving 1st Generation *Daphnia*

Nominal Concentration (mg l <sup>-1</sup> )		Study Day																					
		0	1	2	3	4	5 <sup>a</sup>	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Control	I	50	48	44	43	43	43	29	28	28	28	28	28	28	28	28	28	28	27	27	27	27	27
	II <sup>b</sup>	50	50	50	50	50	30	30	30	30	30	30	30	30	30	30	29	28	28	27	27	27	27
2.5	I	50	50	48	48	48	48	30	30	30	30	30	30	30	29	29	29	29	28	29	29	28	28
	II	50	49	47	47	47	30	30	30	30	30	30	30	30	29	29	28	28	27	27	27	27	27
5.0	I	50	49	49	48	48	48	30	30	30	30	30	30	30	30	30	30	25	25	24	24	24	22
	II	50	50	45	45	45	30	30	30	30	30	30	30	30	29	29	29	29	27	27	26	26	26
10.0	I	50	48	48	48	48	30	30	30	30	29	29	29	28	28	27	25	25	24	25	23	23	
	II	50	50	46	46	44	44	30	30	30	30	29	29	29	29	28	27	26	25	24	24	24	
20.0	I	50	49	49	48	48	48	30	30	30	30	30	30	30	28	21	17	16	16	15	12		
	II	50	48	47	47	46	46	30	28	28	28	28	28	28	20	18	16	13	13	13	11	11	
40.0	I	50	47	47	47	47	30	29	28	28	27	26	19	4	1	1	1	1	1	1	1	1	1
	II	50	48	48	48	47	30	29	28	28	28	25	19	6	5	3	1	0	0	0	0	0	0

<sup>a</sup> numbers in each beaker reduced to 30<sup>b</sup> solvent control

## APPENDIX V : Biological Results

TABLE 15 : Numbers of Young Daphnia

Nominal Concentration (ng l <sup>-1</sup> )		Study Day							Total
		7	9	12	14	16	19	21	
Control	I	502	117	827	702	112	261	469	2990
	II <sup>a</sup>	467	162	807	822	88	392	481	3219
2.5	I	361	217	1021	628	161	168	443	2999
	II	455	34	612	613	174	189	348	2425
5	I	248	314	318	619	132	195	279	2105
	II	317	260	633	555	125	183	398	2471
10	I	249	110	267	487	142	183	268	1706
	II	478	176	699	529	118	242	259	2501
20	I	431	121	646	686	14	34	94	2026
	II	372	115	366	512	17	69	36	1487
40	I	120	401	433	58	0	0	0	1012
	II	137	266	502	165	0	0	0	1070

<sup>a</sup> solvent control

APPENDIX V : Biological Results

TABLE 16 : Daphnia Measurements (mm), Day 9

Nominal Concentration (ng l <sup>-1</sup> )		<u>Daphnia</u>									
		1	2	3	4	5	6	7	8	9	10
Control	I	4.54	4.50	4.61	4.84	4.95	4.61	4.50	4.58	4.69	4.31
	II <sup>a</sup>	5.14	5.10	4.84	4.73	4.58	4.76	4.84	5.03	3.60 <sup>b</sup>	4.99
2.5	I	4.50	4.46	4.20	4.35	4.16	4.46	4.28	4.54	4.39	4.13
	II	4.80	4.54	4.46	4.50	4.69	4.54	5.21	4.54	4.50	4.54
5.0	I	4.61	4.69	4.65	4.69	4.95	4.84	4.58	4.05	5.03	4.09
	II	4.80	4.35	4.73	5.06	4.65	4.80	4.80	4.91	4.31	4.69
10.0	I	4.61	4.50	4.54	4.69	4.39	4.65	4.73	4.80	4.46	4.86
	II	4.28	4.80	4.58	3.79	4.39	4.65	4.54	4.69	4.50	4.35
20.0	I	4.43	3.75	4.16	4.35	4.31	4.43	3.86	4.24	4.28	4.28
	II	4.76	4.69	4.43	4.23	4.73	4.05	4.20	4.73	4.61	4.54
40.0	I	3.83	4.05	4.58	4.46	4.28	4.28	4.09	4.28	4.20	4.24
	II	3.75	4.01	4.13	3.83	3.83	3.90	4.01	4.01	4.09	3.98

<sup>a</sup> solvent control

<sup>b</sup> Daphnia had no spine; not included in statistical analyses

## APPENDIX V : Biological Results

TABLE 17 : *Daphnia* Measurements (mm), Day 21

Nominal Concentration (ng l <sup>-1</sup> )		<i>Daphnia</i>														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Control	I	4.88	4.91	5.10	4.84	4.69	5.25	4.95	4.54	4.73	4.95	4.76	5.25	5.25	5.29	5.06
	II	4.39	4.91	4.91	5.36	4.88	4.88	5.18	4.91	5.18	4.84	5.10	4.76	4.76	4.91	4.84
2.5	I	4.54	4.39	4.95	4.69	4.88	4.65	5.03	4.76	4.61	4.69	4.54	4.69	4.65	4.84	4.61
	II	4.58	4.76	5.40	4.65	5.10	4.50	5.25	5.14	5.29	5.18	5.14	5.06	4.80	4.95	5.25
5.0	I	4.50 <sup>b</sup>	5.18	5.10	5.14	4.91	4.35 <sup>b</sup>	5.06	4.88	4.73	5.14	4.73	4.69	4.95	4.80	5.25
	II	5.06	4.84	5.33	5.10	5.14	5.48	3.79	5.06	5.10	5.18	5.06	4.46	4.91	4.76	4.65
10.0	I	4.84	4.95	5.21	4.73	5.18	5.29	4.84	4.99	4.91	4.80	5.03	4.88	4.50	4.58	4.76
	II	4.91	4.80	5.06	5.14	4.84	4.80	4.99	4.46	4.88	4.46	4.80	4.65	5.06	5.14	4.91
20.0	I	4.58	4.65	4.91	4.80	4.73	4.84	4.99	4.65	4.84	4.95	4.46	4.61	-	-	-
	II	4.99	4.91	4.58	4.61	4.84	4.80	4.65	4.46	4.80	4.65	4.84	-	-	-	-
40.0	I	3.83	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	II	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Nominal Concentration (ng l <sup>-1</sup> )		Daphnia														
		16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Control	I	4.91	4.58	4.61	4.91	4.65	4.76	4.50	4.91	4.98	4.73	4.91	4.84	-	-	-
	II <sup>a</sup>	5.10	4.61	4.99	4.61	4.72	4.58	4.88	4.84	5.03	4.76	4.65	4.61	-	-	-
2.5	I	4.91	4.69	5.18	4.84	4.61	4.76	4.91	4.84	4.91	4.65	4.35	4.76	4.58	-	-
	II	5.03	5.10	5.14	5.29	5.25	4.58	4.84	5.21	4.95	4.28	5.10	5.21	-	-	-
5.0	I	4.69	5.33	4.91	5.10	5.33	4.91	5.14 <sup>b</sup>	-	-	-	-	-	-	-	-
	II	4.99	4.69	4.50	5.18	3.90 <sup>b</sup>	4.69	4.80	4.88	5.03	4.76	5.74	-	-	-	-
10.0	I	4.95	4.80	5.14	5.03	4.95	5.25	5.06	4.88	-	-	-	-	-	-	-
	II	4.88	4.84	4.53	4.91	4.46	4.99	4.91	4.84	-	-	-	-	-	-	-
20.0	I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	II	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
40.0	I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	II	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

<sup>a</sup> solvent control<sup>b</sup> *Daphnia* had no spine; not included in statistical analyses

APPENDIX VI: Effects on Daphnia Transferred to Untreated WaterTABLE 18 : Survival and Number of Young Produced from Daphnia  
Transferred to Untreated Water for 13 days on 6 August 1985

From Nominal Concentration (ng l <sup>-1</sup> )	13 August 1985 (day 7)		16 August 1985 (day 10)		19 August 1985 (day 13)	
	Females	Males <sup>a</sup>	Adults	Young	Adults	Young
Control I	14	6	14	152	14	205
Control II	15	5	15	214	15	244
2.5 I	18	2	18	156	18	341
2.5 II	20	0	20	316	20	433
5 I	20	0	20	271	20	277
5 II	20	0	20	291	19	483
10 I	20	0	19	278	19	365
10 II	20	0	20	221	20	514
20 I	19	0	19	193	19	316
20 II	20	0	20	200	20	297
40 I	20	0	20	218	20	525
40 II	9	0	7	27	6	61

<sup>a</sup> males removed on this day



APPENDIX VI : Effects on Daphnia Transferred to Untreated WaterTABLE 19 : Statistical Analysis of Number of Young Produced from First Instar Daphnia Transferred to Untreated Water for 13 Days

From Nominal Concentration (ng l <sup>-1</sup> )	Total Young Produced		Young per Female per Reproductive Day	
	a	b	a	b
Control	407 A	407 A	4.67 A	4.67 A
2.5	623 A	623 A	5.42 A	5.42 A
5.0	661 A	661 A	5.51 A	5.51 A
10.0	689 A	689 A	5.81 A	5.81 A
20.0	503 A	503 A	4.30 A	4.30 A
40.0	415 A	not included	4.01 A	not included
Standard Error (Single Plot)	216	115	1.48	0.86
Coefficient of Variation	39.4%	20.0%	30.0%	16.7%
LSD P = 5%	530	297	3.63	2.21

<sup>a</sup> statistical analysis included all chambers<sup>b</sup> statistical analysis omitted the 40 ng l<sup>-1</sup> results, as there was a large variation between the two replicates of this treatment